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Screening for Alternative Sources of L-Asparaginase Used in Acute Lymphoblastic Leukaemia (All) Treatment: An *In Silico* Approach

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Abstract

Acute Lymphoblastic Leukemia (ALL) is the most prevalent acute leukemia in children and it also represents a devastating disease when it occurs in adults. Within the United States, the incidence of ALL is estimated at 1.6 per 100 000 population and an estimated 6590 new cases were diagnosed in 2016 alone. The enzyme L-asparaginase (L-Asp) is being used for treatment of childhood acute lymphoblastic leukemia (ALL) for many years because of its unique pharmacological features and historically improved treatment outcomes. As L□asparaginase demonstrates relative substrate specificity and at the same time affects the glutamine metabolism, these may intensify adverse effects including hepatotoxicity, hemostatic disorders and hyperglycemia. That's why alternative L-asparaginase sources are crying needed to tackle the present drawbacks of commercially available L-asparaginase (For example, PEG-asparaginase from Erwiniachry santhemi). The present study planned to suggest an alternative source of L-asparaginase for ALL treatment by in silico analysis, mostly for child patient. The study included phylogenetic tree construction, physiochemical properties analysis, the secondary structure screening and three-dimensional structure prediction of proposed L-asparaginase. After phylogeny analysis and in slico screening of physiochemical and secondary properties, homology modeling of L-asparaginase Shigella boydii (WP_000513786.1) was uggested as the best alternative option for ALL treatment rather than commercially available L-asparaginase sources.

Keywords: L-asparaginase; Acute Lymphoblastic Leukemia (ALL); In Silico Analysis; Plylogeny

Introduction

Acute lymphoblastic leukaemia (ALL) is a malignant disorder of lymphoid progenitor cells characterized by the overproduction and accumulation of cancerous immature white blood cells. While ALL occurs mostly in children (80%), it also represents a devastating phenomenon when it occurs in adults [1]. Though the causation is multifactorial and exogenous or endogenous exposures, genetic susceptibility and chance have roles, but recent outbreaks and frequent incidences make ALL one of the most concerned health issue [2]. For examples, the incidence of ALL is estimated at 1.6 per 100 000 population in the United State and In 2016 alone, an estimated 6590 new cases were diagnosed, with over 1400 deaths due to ALL (American Cancer Society) [1]. The treatment of paediatric acute lymphoblastic leukaemiahas improved dramatically and survival increased from 0-5% in the 1960s to 80-85% nowadays [3].

L‐asparaginase is an important component of induction and consolidation multidrug chemotherapy in children and adults with ALL [4]. L-asparaginase mainly used in amalgamation with vincristine and a glucocorticoid (e.g. Dexamethasone) [5]. The enzyme L-asparaginase (L-Asp) has been commonly used for treatment of childhood acute lymphoblastic leukemia (ALL) for more than 30 years [6-8]. Because of its unique pharmacological features and historically improved treatment outcomes, L-Asp forms an essential part of ALL regimens worldwide [9]. This enzyme impedes tumor cell growth by depriving them from growth related nutrition [10]. In addition, asparaginase is a non-human enzyme which hydrolyses asparagine into aspartic acid and ammonia. Given that leukemic blasts depend heavily on asparagine, deprived of this amino acid, they undergo apoptosis [11].

Three asparaginase preparations are available; the native asparaginase derived from Escherichia coli (E. coli-asparaginase), a pegylated form of this enzyme (PEG-asparaginase) and a product isolated from Erwiniachry santhemi, i.e. *Erwinia asparaginase* [12]. L‐asparaginase demonstrates relative substrate specificity and at the same time affects the glutamine metabolism, which may intensify adverse effects including hepatotoxicity, hemostatic disorders and hyperglycemia [13]. The majority of adverse effects during L‐asparaginase treatment are related with a transient protein inhibition in the liver and pancreas [14]. In earlier published studies on adult patients and children, the presence of E.coli L‐asparaginase antibodies during multiple administrations was reported in 28–96% of patients, while severe allergic reactions were observed in 24% of children and 29% of adults [15]. However, many other adverse effects of L-Asp have been documented, such as coagulopathy, acute pancreatitis, allergic reaction, hyperlipidemia and hyperammonemia [16-19]. The process of treatment in case of adult ALL has been adapted from pediatric protocols. Unfortunately, while long-term survival approaches 90% for standard-risk pediatric ALL, the success rate is much more modest in adults but in case of children it hopes less remedy [20]. So, the present study planned to suggest an alternative source of l-asparaginase for ALL treatment, mostly for child patient by in silico analysis that would be therapeutically administered to result in fewer side effects. This study focused on the phylogenetic analysis for human resembled L-asparaginase source screening, physiochemical properties and three-dimensional structure prediction of proposed L-asparaginase.

Methods

Sequence Retrieval

The sequences of L -asparaginase were retrieved from NCBI (National Center for Biotechnology Information) (https://www.ncbi.nlm.nih.gov). We used L -asparaginase of E. coli (Accesion No: KGM80445) as a template and retrieved 192 sequences including Erwiniachry santhemi as it served as a source of commercial PEG-asparaginase with questionable side effects to patient12 and also Homo sapiens for comparative analysis .

Construction of Phylogeny Tree and Selection of Alternative Clade for L-Asparaginase

Phylogenetics is the science of estimating the evolutionary past, in the case of molecular phylogeny, based on the comparison of DNA or protein sequences. We used MEGA 4.2 software for constructing phylogenetic

tree among the retrieved 192 sequences of L – asparaginase [21,22].

Analysis of Physicochemical Parameters

The different physicochemical properties of L-asparaginase found from the selected phylogentic clade were computed using ExPASy'sProtParam tool and these properties can be deduced from a protein sequence [23,24]. The ProtParam includes the following computed parameters: Molecular weight (M.Wt), theoretical pI, instability index (II), aliphatic index (AI) and grand average of hydropathicity (GRAVY). The computed isolelectric point (pI) will be useful for developing buffer systems for purification by isoelectric focusing method [25]. The instability index provides an estimate of the stability of our protein. A protein whose instability index is smaller than 40 is predicted as stable; a value above 40 predicts that the protein may be unstable [26]. The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). It may be regarded as a positive factor for the increase of thermo stability of globular [27].

The Secondary Structure Analysis

The secondary structure was predicted by 'Self Optimized Prediction Method' with Alignment (SOPMA). SOPMA was employed for calculating the secondary structural features of the selected protein sequences considered in this study [28]. This method calculates the content of α-helix, βsheets, turns, random coils and extended strands. SOPMA is a neural network based methods; global sequence prediction may be done by this sequence method [29].

Homology Modelling

Homology modeling helps to construct a three dimensional structure of proposed protein molecule [30]. Amino acid sequences of Shigella boydii (AC No. WP_000513786.1) and Shigella flexneri k-315 (AC No.EIQ25923.1) were taken for homology modeling and pBLAST was done with PDB as these two L-asparaginase sequences were identified relatively close with E.coli from ProtParam and SOPMA analysis. Homology modeling of L-asparaginase from Shigella boydii (AC No. WP_000513786.1) and Shigella flexneri k-315 (AC No.EIQ25923.1) was done by I-tasser [31,32].

Model Validation

In silico modelling generally have errors in the initial structure. So, validation of algorithm based protein model is an essential part. Different procedures were implied to check the error rate of the models [33-35]. Stereo chemical quality and energy parameters were assessed to determine whether the bond lengths and angles within normal ranges. For this reason, we used RAMPAGE, ERRAT, PROCHECK to extrapolate the validation rate of the protein models [36-39].

Results

Phylogenetic Tree Analysis

Phylogenetic tree was constructed among retrieved 192 sequences of L-asparagine by neighbor joining method. We then manually identified the clade which has E.coli L-asparaginase, clustered with other L-asparaginase amino acid sequences. Selected clade have thirteen amino acid sequences which are closely associated with E.coli L-asparaginase (Figure 1) (WP_040002975.1, WP_029685647.1, WP_051619038.1,WP_024107744.1, WP_012882884.1, WP_019938543.1, WP_025517848.1, WP_051484355.1, WP_000513786.1, WP_039059459.1, EIQ25923.1, WP_000513771.1, EST84617.1) and those sequences were allowed to further analysis.

Screening of alternative L-asparaginase by Physiochemical Characteristics

Physiochemical characteristics of selected sequences were done by ProParam tool. Comparative analysis of selected 13 L-asparaginase sequences was done and L-asparaginase of Shigella boydii (Access No: WP_000513786.1) was found more similar to E.coli (Access no. KGM80445.1). ProtParam analysis presented that L-asparaginase of Shigella boydii was about 51 KDa and theoretical pI is similar to E.coli as 4.92. Instability index indicates half-life of a protein (30) which was 27.47 for Shigella boydii close to 29.46 of E.coli L-asparaginase. In addition, aliphatic index for both proteins were 89.75 and 85.61 respectively (Table 1).

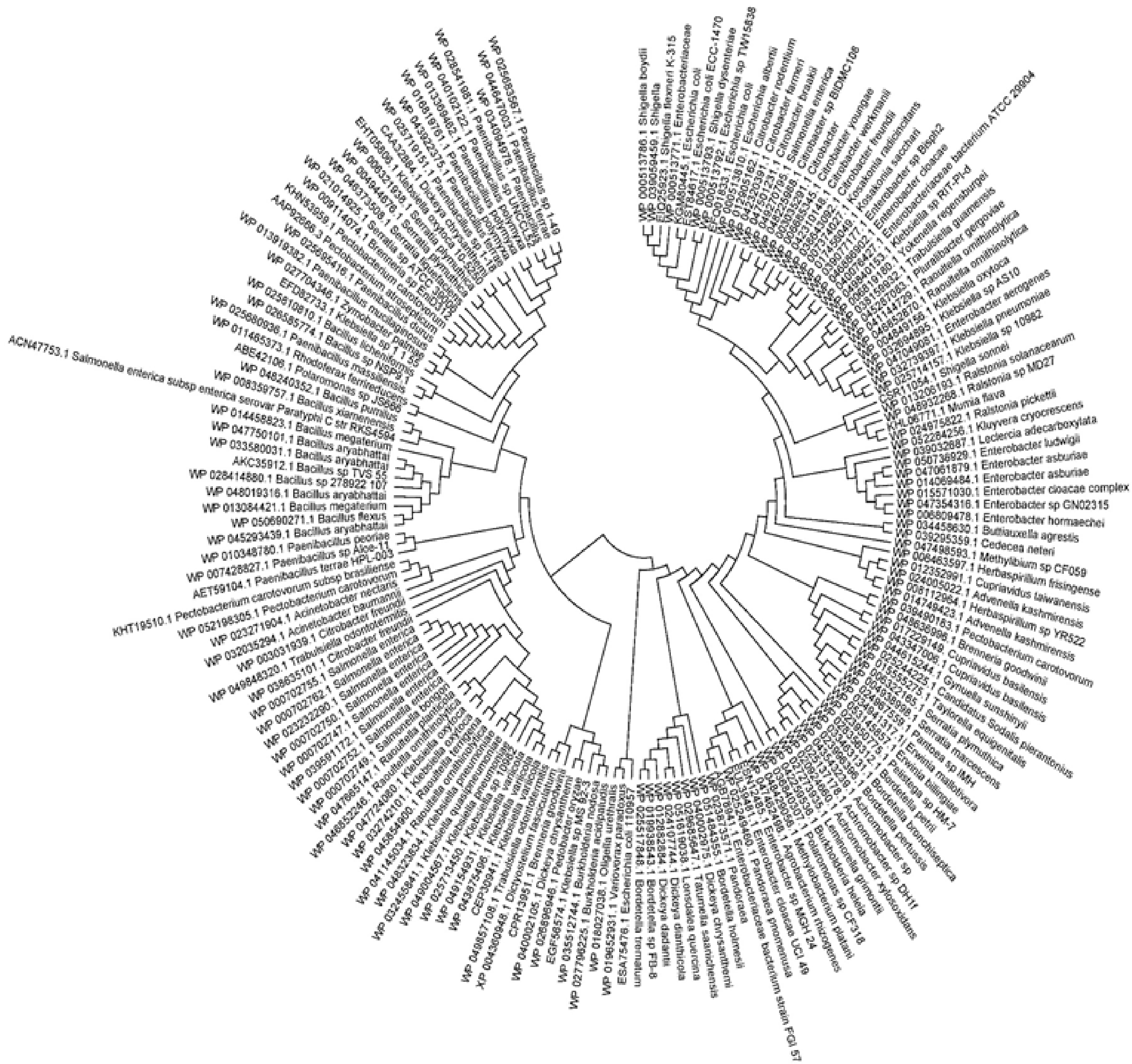


Figure 1: Phylogenetic Tree of L-asparaginase Generated by MEGA 4.2.

Accession number	Molecular weight	Theoretical pI	GRAVY	Instability index	Aliphatic index
WP_040002975.1	32695.9	5.04	0.019	28.92	89.58
WP_029685647.1	36859	4.89	-0.178	36.8	80
WP_051619038.1	41337.7	4.98	-0.071	28.62	83.93
WP_024107744.1	33787	5.32	-0.075	28.62	85.56
WP_012882884.1	38313	5.11	-0.11	28.11	85.29
WP_019938543.1	34005.5	5.45	0.066	20.44	88.2
WP_025517848.1	39017.1	5.38	-0.029	26.68	87.72
WP_051484355.1	42336.7	5.08	-0.052	29.09	85.36
KGM80445.1	46629.9	4.92	0.041	29.46	89.75
WP_000513786.1	51074.8	4.92	-0.03	27.47	85.61
WP_039059459.1	55521.6	4.91	-0.09	26.3	81.74
EIQ25923.1	33326.9	4.88	0.112	33.78	90.69
WP_000513771.1	33287.9	4.93	0.125	32.86	91
EST84617.1	33004.5	4.83	0.094	33.93	90.6
NP_001077395.1	32054.5	5.84	-0.13	16.68	84.87

Table 1: Prot Param analysis of selected amino acid from Phylogenetic Tree.

Screening of Alternative L-Asparaginase From Secondary Structure

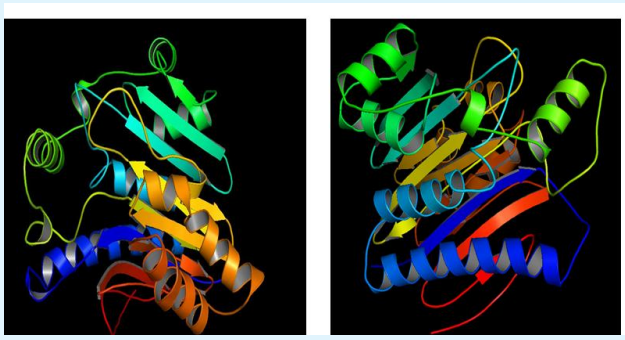
Secondary structure parameters were considered another screening tool for alternation L-asparaginase identification. From the SOPMA analysis we compared eight parameters among the selected thirteen sequences taken from phylogenetic analysis. Here, another source Shigella flexneri k-315 (EIQ25923.1) showed best matched to L-asparaginase from E.coli (KGM80445.1) (Table 2).

Protein	Alpha helix	310 helix	Pi helix	Beta bridge	Extranded strand	Beta turn	Bend region	Random coil
WP_040002975.1	40.58%	0.00%	0.00%	0.00%	19.49%	10.86%	0.00%	29.07%
WP_029685647.1	35.94%	0.00%	0.00%	0.00%	20.62%	13.12%	0.00%	30.31%
WP_051619038.1	38.24%	0.00%	0.00%	0.00%	21.94%	14.73%	0.00%	25.08%
WP_024107744.1	37.89%	0.00%	0.00%	0.00%	19.88%	11.80%	0.00%	30.43%
WP_012882884.1	38.94%	0.00%	0.00%	0.00%	19.31%	11.84%	0.00%	29.91%
WP_019938543.1	37.80%	0.00%	0.00%	0.00%	16.46%	11.28%	0.00%	34.45%
WP_025517848.1	40.00%	0.00%	0.00%	0.00%	18.46%	12.92%	0.00%	28.62%
WP_051484355.1	39.74%	0.00%	0.00%	0.00%	15.38%	12.50%	0.00%	32.37%
KGM80445.1	39.25%	0.00%	0.00%	0.00%	18.38%	12.77%	0.00%	29.60%
WP_000513786.1	37.07%	0.00%	0.00%	0.00%	19.94%	12.46%	0.00%	30.53%
WP_039059459.1	38.01%	0.00%	0.00%	0.00%	18.38%	13.08%	0.00%	30.53%
EIQ25923.1	38.94%	0.00%	0.00%	0.00%	18.38%	13.08%	0.00%	29.60%
WP_000513771.1	38.63%	0.00%	0.00%	0.00%	18.38%	12.15%	0.00%	30.84%
EST84617.1	39.75%	0.00%	0.00%	0.00%	17.67%	12.93%	0.00%	29.65%
NP_001077395.1	28.90%	0.00%	0.00%	0.00%	26.30%	11.04%	0.00%	33.77%

Table 2: Secondary Structure Analysis of Closely Related L-asparaginase.

Homology Modeling of Identified L-Asparagine

After screening from ProtParam and SOPMA, homology modeling was done to extrapolate the three-dimensional (3D) configuration of the two L-asparaginase, Shigella boydii (WP_000513786.1) and Shigellaflexneri k-315 (EIQ25923.1) as 3D structure of those predicted sequences were not available at the Protein Data Bank (PDB). Homology model were conducted by using I-tasser and among the five models for both. Validation software assessed the best structure for both Shigella boydii (WP_000513786.1) and Shigella flexneri k-315 (EIQ25923.1) (Figure 2).



2A 2B

Figure 2: Homology modeling of alternative L-asparaginase.
(2A) Best three-dimensional model of Shigella boydiiand
(2B) Best three-dimensional model of Shigella flexneri k-315

Model Validation

However, in case of Shigella boydii (WP_000513786.1), RAMPAGE, ERRAT, PROCHECK found better result. ERRAT validated models by statistical relation of non-bonded interactions among different atom types based on characteristic atomic interaction20. It assesses overall quality of a model at 0.01 and 0.05 level of significance and presents result as overall quality factor. Standard high resolution structures generally produces values around 95% or higher. Low resolution structures produced values around 91%. Figure 3 illustrate ERRAT score of predicted models of Shigella boydii, which scored overall quality factor more than 90%. Again, the best models form Shigella boydii, which was selected on the basis of RAMPAGE scored more than 84% (Figure 4). This range suggests quality models were predicted by using I-Tasser. PROCHECK tests stereochemical quality of protein structure by evaluating residue-by-residue geometry and overall structural geometry. The best model suggested that more than 77% amino acid residues were in most favored region for these models (Figure 5).

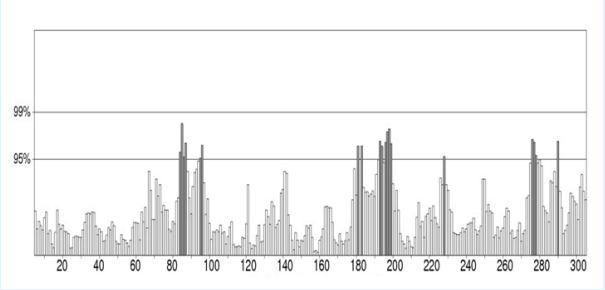


Figure 3: ERRAT result of best models for L-asparaginase of Shigella Boydii.

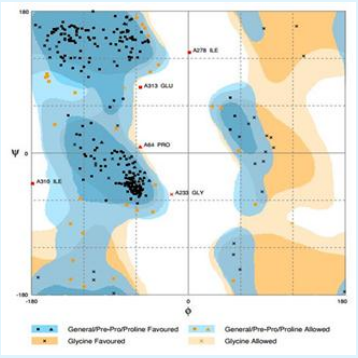


Figure 4: RAMPAGE output of best models for L-asparaginase of Shigella boydii.

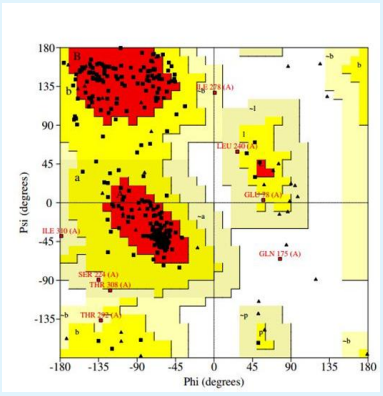


Figure 5: PROCHECK analysis result for best models for L-asparaginase of Shigella boydii.

Color codes are red color- most favorable regions, yellow color region- allowed region and pale yellow-generously allowed region and white color disallowed regions.

Discussion

The retrieved sequences of L -asparaginase were allowed to phylogenetic tree construction. Here, L –asparaginase of Erwiniachry santhemi was included as it served as a commercial source PEG-asparaginase with questionable side effects to patient12. In case of s phylogenetic analysis, screening was done basis on E.coli L-asparaginase as E.coli source is considered much better option for ALL treatment with less side effects36. A clade was identified with 13 L-asparaginase (WP_040002975.1, WP_029685647.1, WP_051619038.1, WP_024107744.1, WP_012882884.1, WP_019938543.1, WP_025517848.1, WP_051484355.1, WP_000513786.1, WP_039059459.1, EIQ25923.1, WP_000513771.1, EST84617.1) including E.coli L-asparaginase.

From the characterization of physiochemical characteristics by ProParam tool, we compared different parameters such as; molecular weight, theoretical pI, GRAVY, instability index and aliphatic index among 13 selected sequences. It was revealed that Shigella boydii (AC No. WP_000513786.1) could be an alternative source of L-asparaginase rather than others selected sources as it was more similar to E.coli (Access no. KGM80445.1) in different physiochemical characteristics. Secondary structure analysis suggested thatanother source Shigella flexneri k-315 (EIQ25923.1) could be better option for ALL treatment as it is much more closed to L-asparaginase from E.coli (KGM80445.1). That's why L-asparaginase from Shigella boydii (AC No. WP_000513786.1) and Shigella flexneri k-315 (EIQ25923.1) were considered as alternative option for L-asparaginase sources rather than Erwiniachry santhemi (commercial source with side effect) in ALL treatment and these were allowed for 3D structure prediction by homology modeling.

After screening of physiochemical and secondary properties, homology modeling of the two L-asparaginase, Shigella boydii (WP_000513786.1) and Shigellaflexneri k-315 (EIQ25923.1) were done as 3D structure of those predicted sequences were not available at the Protein Data Bank (PDB). Validation software assessed the best structure for both Shigella boydii (WP_000513786.1) and Shigella flexneri k-315 (EIQ25923.1) and Shigella boydii was found the best alternative option for ALL treatment.

Conclusion

Cancer in children is rare, although the overall incidence ALL has been slowly increasing. Research on ALL treatment gets more priority in recent couple of years. Commercially available treatments are claimed for major side effects and other obstacles. The study focused on searching alternative sources of L-asperginanage, as it is used in Acute Lymphoblastic Leukemia (ALL) treatment. Though it was done by bioinformatics approach, but now-a-days in silico approaches are being accepted for its huge prescreening strategy before wet lab trials. Here, the study was employed to investigate available treatment methods of Acute Lymphoblastic Leukemia (ALL) and suggested better source of therapeutics used in ALL. The result could be considered for further wet lab study.

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Conflict of Interests

Authors declare that there is no conflict of interest.

References

1. Pui CH (2011) Acute lymphoblastic leukemia. Springer Berlin Heidelberg.

2. Inaba H, Greaves M, Mullighan CG (2013) Acute lymphoblastic leukaemia. The Lancet 381(9881): 1943-1955.

3. Schrappe M, Hunger SP, Pui CH, Saha V, Gaynon PS, et al. (2012) Outcomes after induction failure in childhood acute lymphoblastic leukemia. New England Journal of Medicine 12: 366(15): 1371-81.

4. Avramis VI, Sencer S, Periclou AP, Sather H, Bostrom BC, et al. (2002) A randomized comparison of nativeEscherichia coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. Blood 99(6): 1986-1994.

5. Batool T, Makky EA, Jalal M, Yusoff MMA (2016) comprehensive review on L-asparaginase and its applications. Appl Biochem Biotechnol 178(5): 900-923.

6. Moricke A, Zimmermann M, Reiter A, Henze G, Schrauder A, et al. (1981) Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from to 2000. Leukemia 24(2): 265-284.

7. Tsuchida M, Ohara A, Manabe A, Kumagai M, Shimada H, et al. (2010) Long-term results of Tokyo Children's Cancer Study Group trials for childhood acute lymphoblastic leukemia, 1984–1999. Leukemia 24(2): 383-396.

8. Gaynon PS, Angiolillo AL, Carroll WL, Nachman JB, Trigg ME, et al. (2010) Long-term results of the children's cancer group studies for childhood acute lymphoblastic leukemia 1983-2002: a Children's Oncology Group Report. Leukemia 24(2): 285-297.

9. Pession A, Valsecchi MG, Masera G, Kamps WA, Magyarosy E, et al. (2005) Long-term results of a randomized trial on extended use of high dose L-asparaginase for standard risk childhood acute lymphoblastic leukemia. J Clin Oncol 23(28): 7161-7167.

10. Outschoorn MUE, Pages PM, Pestell RG, Sotgia F, Lisanti MP (2017) Cancer metabolism: a therapeutic perspective. Nat Rev Clin Oncol 14(1): 11-31.

11. Bussolati O, Belletti S, Uggeri J, Gatti R, Orlandini G, et al. (1995) Characterization of apoptotic phenomena induced by treatment with L-asparaginase in NIH3T3 cells. Exp cell Res 220(2): 283-291.

12. Pieters R, Hunger SP, Boos J, Rizzari C, Silverman L, et al. (2011) L-asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. Cancer 117(2): 238-249.

13. Müller HJ, Boos J (1998) Use of L-asparaginase in childhood ALL. Critical reviews in oncology/hematology 28(2): 97-113.

14. Holle LM (1997) Pegaspargase: an alternative? Ann Pharmacother 31(5): 616-624.

15. Woo MH, Hak LJ, Storm MC, Sandlund JT, Ribeiro RC, et al. (2000) Hypersensitivity or development of antibodies to asparaginase does not impact treatment outcome of childhood acute lymphoblastic leukemia. J Clin Oncol 18(7): 1525-1532.

16. Raetz EA, Salzer WL (2010) Tolerability and efficacy of L-asparaginase therapy in pediatric patients with acute lymphoblastic leukemia. J Pediatr Hematol Oncol 32(7): 554-563.

17. Pui CH, Burghen GA, Bowman WP, Aur RJ (1981) Risk factors for hyperglycemia in children with leukemia receiving L-asparaginase and prednisone. Journal pediatr 99(1): 46-50.

18. Carpentieri U, Balch MT (1978) Hyperglycemia associated with the therapeutic use of L-asparaginase: possible role of insulin receptors. The Journal of pediatrics 93(5): 775-778.

19. Roberson JR, Raju S, Shelso J, Pui CH, Howard SC (2008) Diabetic ketoacidosis during therapy for pediatric acute lymphoblastic leukemia. Pediatr blood cancer 50(6): 1207-1212.

20. Scavino HF, George JN (1976) Sears DA Remission induction in adult acute lymphocytic leukemia. Use of vincristine and prednisone alone. Cancer 38(2): 672-677.

21. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24(8): 1596-1599.

22. Hasan M, Joy ZF, Bhuiyan EH, Islam MS (2015) In Silico Characterization and Motif Election of Neurotoxins from Snake Venom. American Journal of Biochemistry & Biotechnology 11(2): 84-91.

23. Hasan M, Hakim A, Iqbal A, Bhuiyan FR, Begum MK (2015) Computational study and homology modeling of phenol hydroxylase: key enzyme for phenol degradation. Int J Comput Bioinfo in Silico Model 4(4): 691-698.

24. Das K, Chakraborty S, Hasan M, Shovo AM (2016) In silico analysis to elect superior bacterial alkaline protease for detergent and leather industries. Journal of Advances In Biotechnology 5(3): 685-698.

25. Sivakumar K, Balaji S (2007) In silico characterization of antifreeze proteins using computational tools and servers. Journal of Chemical Sciences 119(5): 571-579.

26. Guruprasad K, Reddy BB, Pandit MW (1990) Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. Peds 4(2): 155-161.

27. Walker JM (2005) The proteomics protocols handbook. Humana Press, pp: 988.

28. Arora N, Banerjee AK, Mutyala S, Murty US (2009) Comparative characterization of commercially important xylanase enzymes. Bioinformation 3(10): 446-453.

29. Mugilan A, Ajitha MC, Thinagar D (2010) Silico Secondary Structure Prediction Method (Kalasalingam University Structure Prediction Method) using Comparative Analysis. Trends in Bioinformatics 3(1): 11-19.

30. Hasan M, Azim KF, Begum A, Khan NA, Shammi TS, et al. (2019) Vaccinomics strategy for developing a unique multi-epitope monovalent vaccine against Marburg marburgvirus. Infect Genet Evol 140-157.

31. Zhang Y (2008) I-TASSER server for protein 3D structure prediction. BMC bioinf ormatics 9(1): 40.

32. Sippl MJ (1993) Recognition of errors in three-dimensional structures of proteins. Proteins 17(4): 355-362.

33. Al-Hakim, Hasan M, Ali H, Rabbee MF, Joy ZF, et al. (2015) In-silico characterization and homology modeling of catechol 1, 2 dioxygenase involved in processing of catechol-an intermediate of aromatic compound degradation pathway. Glob J Sci Front Res Bio-Tech Genetics 15(1): 1-13.

34. Hasan M, Ghosh P, Azim KF, Mukta S, Abir RA, et al. (2019) Reverse vaccinology approach to design a novel multi-epitope subunit vaccine against avian influenza A (H7N9) virus. Microb patho 130: 19-37.

35. Colovos C, Yeates TO (1993) Verification of protein structures: patterns of nonbonded atomic interactions. Protein Science 2(9): 1511-1519.

36. Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Crystallography 26(2): 283-291.

37. Lovell SC, Davis IW, Arendall III WB, De Bakker PI, Word JM, et al. (2003) Structure validation by Cα geometry: ϕ, ψ and Cβ deviation. Proteins: Structure, Function, and Bioinformatics 50(3): 437-450.

38. Guruprasad K, Reddy BB, Pandit MW (1990) Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. Protein Engineering Design & Selection 4(2): 155-161.

39. Terwilliger T, Abdul-Hay M (2017) Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer Journal 7(6): 577.